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Original article

Measuring local immunoglobulin E in the inferior turbinate nasal mucosa in patients with allergic rhinitis

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ABSTRACT

Background: Studies show that immunoglobulin E (IgE) is produced in the local nasal mucosa in allergic rhinitis patients. However, no study involved the measurement of IgE levels in the local nasal mucosal tissue in allergic rhinitis patients. This study aimed to measure the local IgE levels in the nasal mucosal tissue and to compare the levels of total IgE and specific IgEs in the serum and the inferior turbinate nasal mucosa in allergic rhinitis patients using the AlaSTAT 3gAllergy assay (Siemens Healthcare Diagnostics AG, Erlangen, Germany).

Methods: Total IgE antibodies and allergen-specific IgE antibodies in each sample of nasal mucosal tissue from 11 allergic rhinitis patients were measured with the AlaSTAT 3gAllergy assay. We compared the levels of total IgE and IgEs specific for house dust (HD), mites, and cedar pollen in the serum and the inferior turbinate.

Results: The total IgE levels and the cedar pollen-specific IgE levels in the inferior turbinate mucosal tissue correlated significantly with their respective levels in serum. The HD- and mite-specific IgE levels in the inferior turbinate mucosal tissue did not correlate significantly with their respective levels in the serum.

Conclusions: Our results evaluating the correlations between nasal mucosal and serum levels of antigen-specific IgE indicate that IgE produced in the nasal mucosa affects the IgE levels in the serum, especially the cedar pollen-specific IgE.

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Introduction

Immunoglobulin E (IgE) in the local nasal mucosa in allergic rhinitis patients has been attracting attention recently. Studies have shown that IgE is produced in the local nasal mucosa in allergic rhinitis patients^{1–4}; hence, we aimed to measure the local IgE levels in nasal mucosal tissues. Several investigators have measured IgE levels in the local nasal mucosa using mucosal brush biopsy⁵ and nasal lavage^{1,6–8} and presented some reports of measurement of IgE in the local paranasal mucosal tissue in chronic rhinosinusitis^{9,10}; however, no studies involved the measurement of IgE in the local nasal mucosal tissue in allergic rhinitis patients. We compared

the levels of total immunoglobulin E (IgE) and specific IgEs in the serum and the inferior turbinate nasal mucosa in allergic rhinitis patients using the AlaSTAT 3gAllergy assay (Siemens Healthcare Diagnostics AG, Erlangen, Germany).

Methods

Nasal mucosal tissue samples from the inferior concha were collected from 11 patients (5 patients with seasonal allergic rhinitis, 4 patients with persistent allergic rhinitis, and 2 patients with mild allergic symptoms) who had undergone an inferior turbinatectomy at the department of otorhinolaryngology in Toho University Medical Center, Sakura Hospital, between June and November 2013. We measured serum IgE levels for each patient. A diagnosis of allergic rhinitis was made, considering the presence of nasal symptoms (e.g., sneezing, nasal obstruction, and running nose) and clinical findings such as pale nasal mucosa and serum IgE positivity. None

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of the patients had other allergic diseases including asthma and atopic dermatitis; however, two patients had asthma as a complication. We did not collect nasal and serum samples during the cedar pollen season.

We recognized a slight shadow in the maxillary and ethmoid sinuses in two cases of persistent allergic rhinitis, but computer tomography did not reveal mucous membrane thickening in the other nine cases.

Total and specific IgE levels in the mucosal tissue were measured using the AlaSTAT 3gAllergy assay. Inferior concha mucosa samples harvested during surgery were weighed and then homogenized with 1 mL of 0.1 M PBS (Siemens diluent; Siemens Healthcare Diagnostics AG, Germany). Each sample was subsequently homogenized with a mechanical homogenizer at 1000 rpm for 5 min. The homogenized samples were then centrifuged at 3000 rpm for 5 min, and the fractionated supernatants were used as measurement samples. The total IgE antibody level in each sample was measured using the AlaSTAT 3gAllergy assay and expressed as international units per milliliter per gram ($\text{IU mL}^{-1} \text{g}^{-1}$). The levels of total IgE and antigen-specific IgEs (specific to mites, house dust [HD], cedar pollen, fungus, enterotoxin A, and enterotoxin B) were measured using the AlaSTAT 3gAllergy assay and expressed as international units per milliliter per gram ($\text{IU mL}^{-1} \text{g}^{-1}$).

The level of IgE in the inferior concha mucosa of the normal controls was considered background level, which was then subtracted from each measurement to yield the final results. We then calculated the average level of each specific IgE in the nasal mucosa and serum in order to determine the differential presence of IgE species in the inferior concha mucosa compared with serum.

To confirm the validity of our results, we determined the coefficients of correlation between the serum and mucosal tissue levels of the total and HD-, mite-, and cedar pollen-specific IgEs.

We also estimated the eosinophil count in the inferior concha mucosa. We determined the eosinophil count for each specimen at $400\times$ magnification by using the five sites with the highest eosinophil count in accordance with the method described by Sakuma and Ishitoya.¹¹ Subsequently, the average eosinophil count for the top three sites in terms of eosinophil count was calculated and used as the eosinophil count for each tissue sample. In addition, we determined the percentage of eosinophils in the total number of white blood cells in blood.

To confirm the relationship between the eosinophil counts and IgE levels, we checked the coefficients of correlation between the mucosal eosinophil count and mucosal tissue levels of the total IgEs.

This study was approved by the ethical review board of the Toho University, Sakura Medical Center (2012-103).

Results

The average levels of the specific IgEs in the inferior turbinate and serum were, respectively, $13.22 \text{ IU mL}^{-1} \text{g}^{-1}$ and 60.29 IU mL^{-1} (mites), $7.89 \text{ IU mL}^{-1} \text{g}^{-1}$ and 32.70 IU mL^{-1} (HD), and

$4.94 \text{ IU mL}^{-1} \text{g}^{-1}$ and 21.57 IU mL^{-1} (cedar pollen). Of the 3 specific antigens assayed, the mite-specific antigen level was the highest and the cedar pollen-specific antigen level was the lowest in the inferior turbinate mucosa and serum, although the differences were not significant (Fig. 1).

The total IgE levels in the inferior turbinate mucosal tissue samples correlated significantly with those in the serum samples ($r = 0.608$, $P < 0.05$) (Fig. 2). The HD-specific and mite-specific IgE levels in the inferior turbinate mucosal tissue samples did not correlate significantly with their respective levels in the serum samples ($r = 0.428$ and 0.382 , respectively) (Fig. 3, 4). However, the cedar pollen-specific IgE levels in the inferior turbinate mucosal tissue samples correlated significantly with the cedar pollen-specific IgE levels in the serum samples ($r = 0.609$, $P < 0.05$) (Fig. 5).

Fungus-, enterotoxin A-, and enterotoxin B-specific IgEs were detected in the inferior turbinate mucosa sample of only one patient.

The percentage of blood eosinophils was within the normal range, except in one case, which was complicated with asthma (normal $<8\%$). There were no significant correlations between the mucosal eosinophil counts and mucosal tissue levels of total IgE. The results are shown in Table 1.

Discussion

Several investigators have measured IgE levels in the local nasal mucosa using mucosal brush biopsy³ and nasal lavage,¹ although not in the nasal mucosal tissue itself. Studies have shown that IgE is produced in the local nasal mucosa in allergic rhinitis patients and patients with chronic sinusitis with nasal polyp^{1,2}; therefore, we measured the local IgE levels in the nasal mucosal tissue itself.

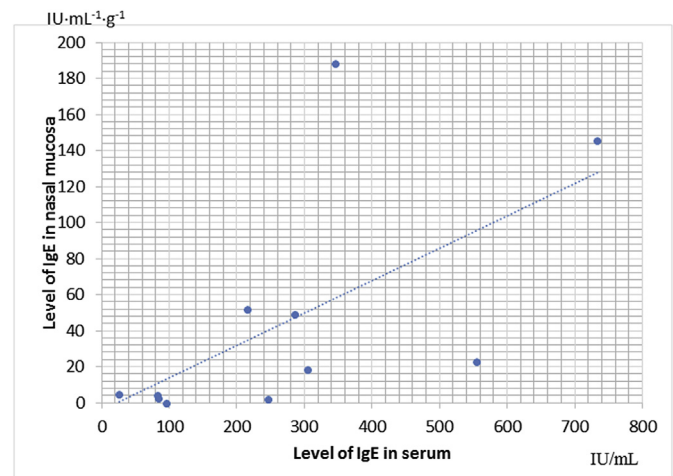


Fig. 2. Total IgE levels in the serum and nasal mucosal tissue samples ($r = 0.608$, $P < 0.05$).

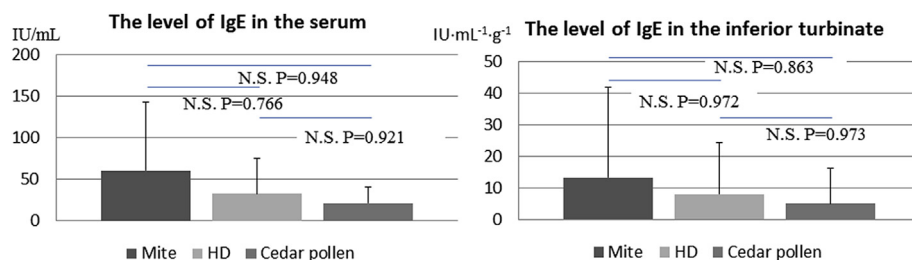


Fig. 1. Average specific IgE levels in the serum and inferior turbinate samples. N.S., not significant.

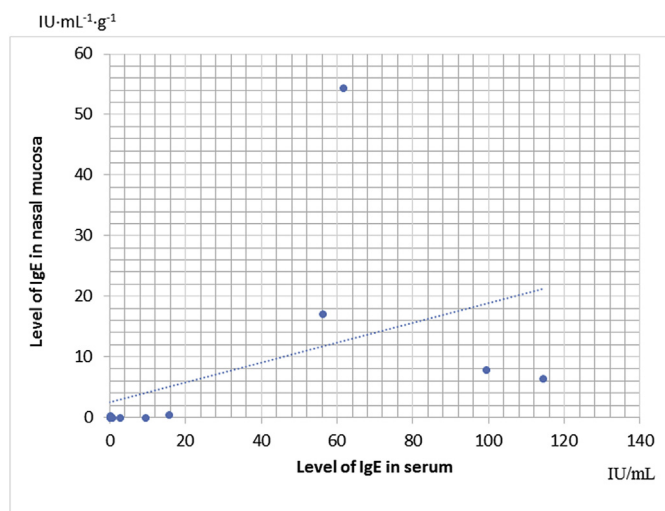


Fig. 3. House Dust-specific IgE levels in the serum and nasal mucosal tissue samples ($r = 0.428$).

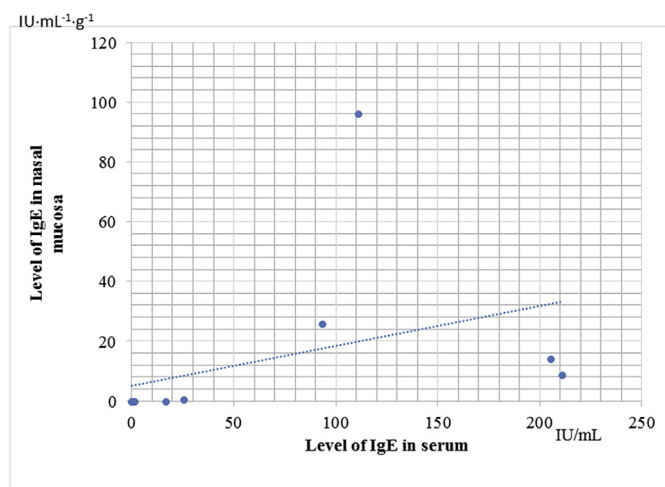


Fig. 4. Mite-specific IgE levels in the serum and nasal mucosal tissue samples ($r = 0.382$).

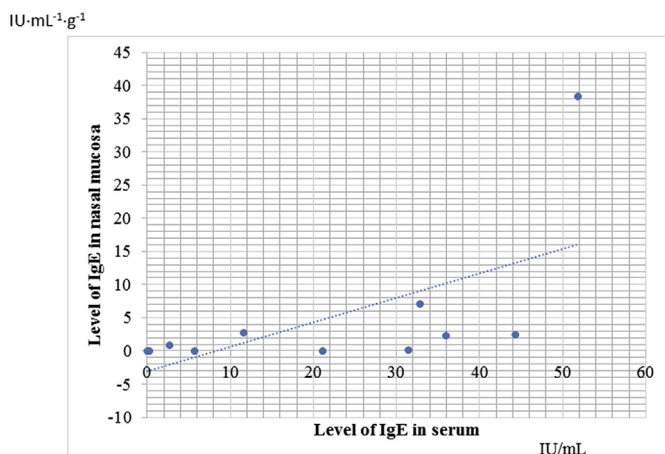


Fig. 5. Cedar pollen-specific IgE levels in the serum and nasal mucosal tissue samples ($r = 0.609$, $P < 0.05$).

The AlaSTAT 3gAllergy assay, which uses beads in the liquid phase, can measure low levels of IgE antibodies (detection limit, 0.1 IU/mL). In the current study, we used the AlaSTAT 3gAllergy assay to measure the local total and antigen-specific IgE levels in the nasal mucosa.

The antigens of the present IgE, from the most abundant to the least, were mite, HD, and cedar pollen, both in the serum and inferior turbinate mucosal tissue of allergic rhinitis patients. These results are similar to those of another Japanese study¹² and therefore likely reflect the accurate situation of Japanese allergic disease.

The production of specific IgE in the nasal mucosa in local allergic rhinitis has been pointed out in previous reports.^{1,2} Yoshida reported that the proportions of specific IgE in the nasal lavage fluid were remarkably higher than in serum in most seasonal rhinitis patients, which strongly supported the predominant in situ production of the specific IgE and its subsequent dilution upon entering the systemic circulation.⁶ Based on these results, it is thought that the mite-, HD-, and cedar pollen-specific IgEs found in the nasal mucosa are also produced there.

In this study, the total IgE levels in the inferior turbinate mucosal tissue correlated significantly with those in the serum. This suggests that IgE production in the nasal mucosa may affect serum IgE levels.

We previously reported that there was no correlation between the circulating levels of total IgE and the count of IgE-positive cells in the paranasal mucosa in chronic sinusitis patients with concurrent asthma.¹³ We think that an exogenous allergy antigen will affect serum IgE levels in allergic rhinitis patients to a greater extent than in chronic sinusitis patients with concurrent asthma.

The HD- and mite-specific IgE levels in the inferior turbinate mucosal tissue did not correlate significantly with the HD- and mite-specific IgE levels in the serum, indicating that these IgEs are produced not only in the nasal mucosa but also in other parts of the body. Two patients who experienced asthma as a complication had persistent allergic rhinitis and the HD- and mite-specific IgE levels in the serum were high. HD and mite-specific IgE may be produced in the lungs and bronchi. It is also possible that they may be produced by the skin.

Therefore, the HD- and mite-specific IgEs produced in the inferior turbinate mucosal tissue do not affect the HD- and mite-specific IgE levels in the serum. However, most of the cedar pollen-specific IgE may be produced in the nasal mucosa; therefore, the cedar pollen-specific IgE levels in the inferior turbinate mucosal tissue correlated significantly with the cedar pollen-specific IgE levels in the serum. In addition, in case of cedar pollinosis, most allergic reaction is sensitized in the nasal cavity. These results agree with the results of Sakaida's report.⁷

We cannot deny that serum IgE may influence local IgE levels. If this is true, local IgE levels should correlate with the serum levels of all antigens including HD and mite antigens. However, in our study, local IgE levels did not correlate with serum levels of IgE against HD and mite antigens. Therefore, it might be a low possibility that serum IgE levels influence local IgE levels in such cases.

Maeda performed a clinical study of Japanese cedar pollen-induced asthma.¹⁴ He reported on one case of cedar pollen-induced asthma with the positive results in the bronchial provocation test with cedar pollen. Based on this report, cedar pollen-specific IgE may be produced in the bronchi.

The percentage of blood eosinophils was within the normal range, except in one case, which was complicated with asthma. Examination of the nasal cavity of this patient revealed normal findings, and we believe that intractable eosinophil-related inflammation such as eosinophilic rhinosinusitis was not possible in this case. Fukushima showed a significance association between the IgE levels and eosinophil counts in the nasal discharge samples of Japanese cedar pollinosis patients.¹⁵ In our study, there was no

Table 1

The results of 11 allergic rhinitis patients.

Inferior Turbinate										
Case		Eosinophils	Total IgE $\mu\text{g mL}^{-1} \text{g}^{-1}$	Dermatophagoides $\text{IU mL}^{-1} \text{g}^{-1}$	House Dust- Japan $\text{IU mL}^{-1} \text{g}^{-1}$	Japanese Cedar $\text{IU mL}^{-1} \text{g}^{-1}$	Fungi $\text{IU mL}^{-1} \text{g}^{-1}$	Enterotoxin A(S.Aureus) $\text{IU mL}^{-1} \text{g}^{-1}$	Enterotoxin B(S.Aureus) $\text{IU mL}^{-1} \text{g}^{-1}$	Cockroach $\text{IU mL}^{-1} \text{g}^{-1}$
1	Seasonal	1	18.45	8.864	6.495	7.135	0	0	0	
2	Persistent	9	49.3	25.7	17.04	0.99	0	0	0	
3	Persistent	3	52.02	0	0.293	38.48	0	0	0	
4	Persistent	2	145.47	0	0	0	0	0	0	
5	Mild	19.6	188.52	96	54.51	0	1.08	0.21	0	
6	Persistent + Asthma	88.3	4.05	0.64	0.53	0	0	0	0	0
7	Seasonal	2	4.75	0	0	2.75	0	0	0	0
8	Seasonal	2	2.57	0	0	2.5	0	0	0	0
9	Mild	3.3	1.93	0	0	0.186	0	0	0	0
10	Persistent + Asthma	3.6	22.56	14.24	7.9	2.35	0	0	0	0
11	Seasonal	2.3	0	0	0	0	0	0	0	0
Average		12.37	44.51	13.22	7.89	4.94	0.1	0.02	0	0

Serum										
Case		Eosinophils %	Total IgE $\mu\text{g mL}^{-1}$	Dermatophagoides IU mL^{-1}	House Dust- Japan IU mL^{-1}	Japanese Cedar IU mL^{-1}	Fungi IU mL^{-1}	Enterotoxin A(S.Aureus) IU mL^{-1}	Enterotoxin B(S.Aureus) IU mL^{-1}	Cockroach IU mL^{-1}
1	Seasonal	2.1	330	210.8	114.4	32.8	0	0	7.16	
2	Persistent	2.9	320	93.3	56.1	2.58	0	0.109	0.644	
3	Persistent	2.3	240	0	0.104	51.8	0	0.28	0.282	
4	Persistent	3.1	1900	0.141	0	0.153	1.27	0	0.319	
5	Mild	3.5	300	110.8	61.6	0	2.536	0.498	0	
6	Persistent + Asthma	6.2	74	25.3	15.64	5.69	0	0	0.32	0
7	Seasonal	1.6	21	0	2.54	11.6	0	0	0.1	0.2
8	Seasonal	2.2	75	0	0	44.2	0	0	0	0
9	Mild	1.3	230	1.18	0.59	31.4	0.129	0	0	0.569
10	Persistent + Asthma	8.7	620	205	99.4	35.9	0.36	0.659	0.251	0
11	Seasonal	4.3	86	16.66	9.31	21.1	0	0.103	0.1	0.119
Average		3.47	381.45	60.29	32.7	21.57	0.39	0.15	0.83	0.15

significant correlation between mucosal eosinophil count and mucosal tissue levels of total IgE.

Some authors reported that a positive nasal allergen provocation test result along with a negative skin prick test and specific IgE test results can confirm local allergic rhinitis.^{1,16,17} The method of measuring local IgE levels in the inferior turbinate nasal mucosa may be useful for the diagnosis of local allergic rhinitis, because the levels of total IgE in the nasal mucosa can be measured using this method. In the present study, there was no case of local allergic rhinitis, because no patient had a negative result for serum IgE and positive result for nasal mucosa IgE for any antigens.

Conflict of interest

The authors have no conflict of interest to declare.

Author's contributions

YO performed the research and data analysis, and wrote the paper. YI, NH, and MK conducted the pathology portion of this research. TS and TF measured the IgE levels in the inferior turbinate nasal mucosa. HB and MS contributed to the data analysis and the preparation and revision of the manuscript.

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